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AN APPARATUS AND TECHNIQUE FOR THE FORCIBLE SILKING OF SPIDERS[1]

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ABSTRACT

Apparatus and auxillary equipment for the forcible silking of spiders are described and illustrated. These facilitate the identification of the glandular sources of the fibers, allow for their localized isolation on a wind-up mandrel, and make possible their removal as continuous lengths or toroidal bundles for further study. Detailed descriptions are given for preferred techniques.

BACKGROUND

The silk fibers produced from the major ampullate gland systems of orb-web-spinning spiders have been the main subject of a continuing program of research. It is generally agreed that this pair of fibers is found in the orb web and is the essential constituent of the dragline and the trailing silk. It cannot be known who first discovered that such silk also can be forcibly drawn from immobilized spiders, but Wilder (1868) described the method. It is the normal means of securing large samples (Zemlin 1968). Work (1976) found that in such an operation minor ampullate silk fibers may also be taken inadvertently. Subsequent papers (Work 1977a, 1977b, 1978, 1981a, and 1981b) emphasized the need for and means of differentiating between these two types of fibers, similar in some properties but quite different in others. Thus, very early in the present investigation it became imperative to develop apparatus wherewith one or the other or both fibers could be secured with certainty and do this under controlled conditions. It follows that the silk taken from spiders should be recoverable with reasonable sureness and ease. Also, it should be possible to obtain and associate the portion of a total sample with the

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conditions under which it was produced, when those conditions were changed during a silking, as for example, velocity of silk withdrawal. Finally, it has been necessary to secure samples large enough for X-ray diffraction measurements and amino acid assays (which will be the subjects of a future paper). The following section (R. W. W.) will describe the method of forcible silking and will refer to the mechanical device used to the degree necessary for clarity; the next section (P. D. E.) will describe the apparatus in sufficient engineering detail so as to make possible its duplication.

METHODS

Although one investigator can manage the forcible silking operation, a second observer should be available if this is possible. The wind-up mandrel (in this case expandable) upon which the silk fibers are to be accumulated, and its driving mechanism are seen, A and B respectively, in Figure 1. Certain auxillary equipment is needed, much of which is commonly available in laboratories, and most items of which are illustrated in Figure 1. Of these, the most essential item is a Greenough type, stereo microscope, C, equipped with a zero objective, 10X oculars and a 1X to 7X zoom feature, and mounted on a cantilever arm. One, preferably two, microscope illuminators, D, are needed, as is the usual collection of manipulative tools, of which a micro dissecting set (Clay-Adams), E, is preferred. A ready supply of about 1″-2″ lengths of narrow self-adhesive tape, F, should be at hand. While it is not imperative that carbon dioxide be available for anesthethization, (out of Figure 1, to the left) its use reduces the hazard of injury to spiders. A bubbler, G, in the supply line provides for visual observation of flow rate, and a two way stopcock, H, on its exit side allows the gas to be directed to either of the two places where it will be needed. One of these is the "operating table", I, being a plenum chamber with a porous plastic top surface; the other any simple glass jar, J, with a few holes punched in its metal cover. Finally, the observer will find useful a head-band mounted jeweler's loupe, K, (3 or 4X

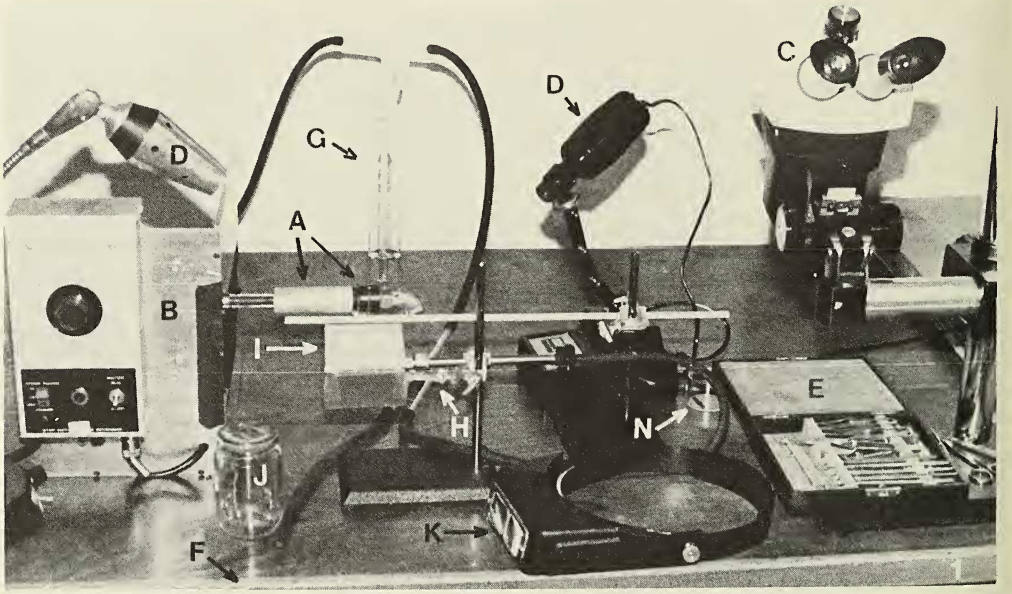


Fig. 1.—Layout of equipment for the forcible silking of spiders, as detailed in the text.

mag.), which may be pivoted quickly into position when needed, or raised vertically when not required, or when looking through the microscope. With items A to K made ready, the microscope is moved into position and focused, the lights brought to bear on the operating table, the forcible silk guides, L, each consisting of two staggered needles embedded in a wooden supporting rod, are positioned, as may be seen in Figure 2. The microscope body is then rotated so as to provide unimpeded access to the operating table. The spider is placed in a clean jar, J, and carbon dioxide supplied by the hose, M, is directed into the jar through one of the holes in its cover. When the spider becomes quiescent, any silk fibers entangling it and cemented to the inside surface of the container are cut free, care being taken to note the presence of trailing silk leaving its body. It is transferred, ventral side up, to the porous plate of the operating table. Any remnants of silk which will interfere with the pinioning of legs, other than the trailing silk, usually held by a fourth leg, are cut loose and removed with tweezers.

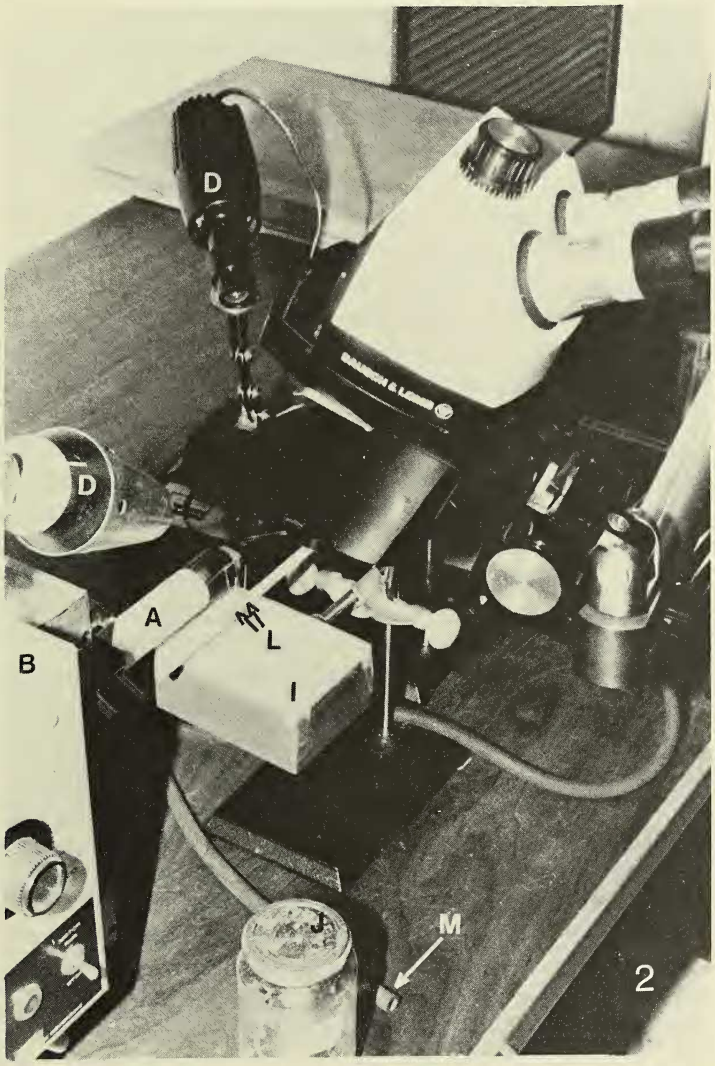


Fig. 2.–"Operating table" and auxillaries prior to pinioning of spider, as detailed in text.

Whether the spider is placed posterior toward or away from the observer will depend on experience with species: this investigator prefers the latter position. The same applies to the conditions for and the order of pinioning the legs with short lengths of self-adhesive tape. But, it is essential that this be done quickly, as the effect of the carbon dioxide is rapidly lost. It is here that four hands are better than two. Each leg can then be fully extended with tweezers while the cephalothorax is gently held in place, and a first strip of tape placed across the leg. Sometimes two legs can be trapped with one length of tape. But if the spider becomes suddenly and violently active during this pinioning, and appears to be in danger of damaging itself by loss of legs, it is advisable to envelop it with a loose wad of facial tissue. It can then be freed from the tape already in place, reanesthetized, and the pinioning repeated. If one of the fourth legs holds the trailing silk in its tarsal claw, it is best to pinion this one last, after cutting the silk free and taking care not to trap the end under the tape. With the spider now prevented from struggling free, it may be expedient to reposition or add additional lengths of tape to any leg that may appear to be insecure.

When the effect of the carbon dioxide is dissipated, the observer will probably receive a hint as to what is to be expected. Some spiders will remain quiescent; others will struggle. It is possible that the behavior is species related; *Araneus diadematus* Clerck and *Argiope aurantia* Lucas behaved in the former manner; *Neoscona hentzi* (Keyserling) in the latter in the present study, for example. Each type will require the use of different methods, and the non-resisting will be considered first. If the trailing silk is obvious, it may be possible to grasp it with tweezers without swinging the microscope into position to locate it. If not, and even if under magnification no silk can be seen emerging from between the folded inward anterior and posterior spinnerets, an attempt must be made to stimulate its start. Often this can be done by gently inserting the end of a hooked micro dissection needle between the anterior spinnerets and stroking their piriform bearing surfaces. This may trigger the deposition of piriform cement which may in turn trap one or more of the major or minor ampullate fibers. Whatever is secured is drawn slowly away from the abdomen until a length is available that can be grasped with tweezers.

The chances are that the observer will not know what is being carried away and brought to the mandrel. But having secured a connecting line of silk from the body of the spider to the mandrel it is now time to attempt to determine what is ready to be wound. While observing the spinneret area through the microscope, the mandrel is rotated slowly by hand to make the connecting line taut. At about 20 to 30X magnification, from one to four entities will be seen, although rarely, even a sheet of fine fibers will be found.

If there are four, the two anterior will be seen to be larger that the two more posterior, indicating the presence of major and minor ampullate pairs, respectively. With three, sizes may indicate which one is missing. When there are two, the greater probability is that they are major ampullate, but this is far from being a certainty. Their presence can be confirmed by starting the motor drive and pulsing the speed of the mandrel, while watching the anterior spinnerets. If these respond by becoming more erectile and then less so, as a function of velocity of silk removal, the major ampullate spigots on these spinnerets is supplying the material. But this does not prove that the minor ampullate pair is not also being taken, since each of the pair may have become attached in line contact with its corresponding major. With some species the mandrel speed may be increased to a point where the sources of the fibers can be seen with very strong vertical illumination and 50 to 70X magnification. Whatever the situation, when in doubt it is best to make use of the flexibility of the silking equipment to isolate the fibers being secured. But before this step is described it is necessary to return to the problems posed by the spiders which react negatively to pinioning and forcible silking.

No facile descriptions can be given as to what an investigator should best do to secure the desired sample in such a situation. Sometimes the difficulties can be solved by sheer presistence. If not, the spider may require complete anesthetization. But this is not an easy way out. In order to start the forcible silking of such a spider, the silk, as yet of undefined origin, must be where it can be grasped with tweezers. The inert animal does not respond to the stroking of the piriform spools. Furthermore, as has been already reported (Work 1976), the physical properties of fibers taken from a fully anethetized spider may differ from those otherwise secured. Sometimes a compromise condition may be achieved by the use of carbon dioxide on a periodic or pulsing basis. This is aided by the use of a specially constructed cell.

Carbon dioxide may be administered by flooding the plenum chamber with it and allowing it to rise through the porous plate to which the spider is pinioned. Being heavier than air, this gas then tends to surround the spider. A glass cell inverted over it helps to retain the gas and gives the observer additional control over conditions. But the construction of such a device, although simple, necessitates the skill of a glassblower. It consists of a piece of glass tubing of diameter somewhat larger than the leg span of the pinioned spider. This is cut to a length greater than the distance between the porous plate and the top most point of the spider's body. At one place this cylinder is cut from one end almost to the other, making a slot about 1 mm wide, which is then fire polished. A piece of optical glass, as for example a double size microscope slide is then cemented to the upper end of the cell and the excess trimmed away with a diamond saw. This cell can be placed over the spider after the silk is fastened to the mandrel, and then observations can be made by means of the microscope through the optical glass, with the silk being led to the mandrel via the slot. Such a cell, N, is partially hidden, but is in Figure 1.

The silk having been started from the spider by one means or another, secured to the mandrel and then tentatively identifed as to source, can not be collected. Guide pairs of needles may be spaced as desired in a small wooden dowel rod, placed parallel and adjacent to the mandel. Each fiber entity desired as a separate sample is then led between the pair of guide needles which will place it on a preselected position on the mandrel. The capacity of this last to be moved horizontally at a uniform rate as it rotates or remain in a fixed position, allows for the primary samples to be placed as a helix or in a piled-up bundle, or the former may preceed or follow the latter, as desired.

In Figure 3 a pinioned *Argiope argentata* (Fabricius) is seen from which major and minor pairs of ampullate silk have been started to be wound on an expandable mandrel. Arrow, P, indicates the band of both fibers (to be discarded later) placed at the beginning of the operation, during which identification had been made. The silking was then stopped, the minor pair transferred between the left guide needles and the major pair allowed to remain between the right guide needles. The silking was then restarted as a very slow (1 cm/sec) rate, and at the same time the dowel was moved slowly from left to right. This placed a helix of each type of silk on the mandrel, from which secondary samples later could be rewound for positive identification or any other study that might be desired. The helices are identified by brackets Q and R, respectively. At the right side of each helix the large samples have been started. Figure 3 also illustrates the use of a piece of ¼″ dowel rod, S, to raise and immobilize the spider's abdomen. The 2-way stopcock of the carbon dioxide system is seen in the background.

It is essential that the primary sample be a helix if the investigator wishes to make a positive identification of a bundle to follow, or if a number of primary samples are needed for individual study. The former, of course, is essential if an X-ray diffraction or

amino acid investigation is involved. But if only a smaller amount is required, then the expandable type of mandrel is best replaced with a simple cylindrical one (painted black for contrast). This allows for the advancing mechanism of the machine drive to produce a uniformly spaced helix without requiring the investigator to make it in a less satisfactorily controlled manner.

The transfer of samples from the helix can be started at any selected point by first placing tiny tabs of self-adhesive tape on the primary sample at each side of that place. It is then cut with a micro scalpel between the tabs, one of which will remain in place to prevent a loose end from interfering with the backwinding. The other tab, with the end of the silk sample adhering to it, is grasped with tweezers and as the mandrel is back rotated by hand, the transfer can be made. In the event that the sample is broken or the end being manipulated pulls free of the tab, these being not unusual happenings, the lost end can be found or a new one started from the helix, which is a virtual impossibility from a bundle.

It has been found that with those spiders which do not resist forcible silking, winding can be done at about 3 m/min. for periods of ten to twenty minutes. During this time the spider appears to be capable of supplying a continuous flow of progenitive polypeptide and in turn allow it to be converted to silk fibers by the drawing action of the forcible silking operation. (As a first approximation, under these conditions a mature female *Araneus diadematus* Clerck, furnishing a pair of major ampullate fibers, each of 3μm diameter, will supply slightly less than 0.06 mg/min. of primary sample). Throughout this entire silking the process must be observed by means of the microscope. If and when there is any disturbance in the spinneret area, chiefly the back and forth rubbing together of the piriform bearing surfaces of the anterior spinnerets, it is necessary to stop the mandrel immediately. If there is any question in the observer's mind that a change could

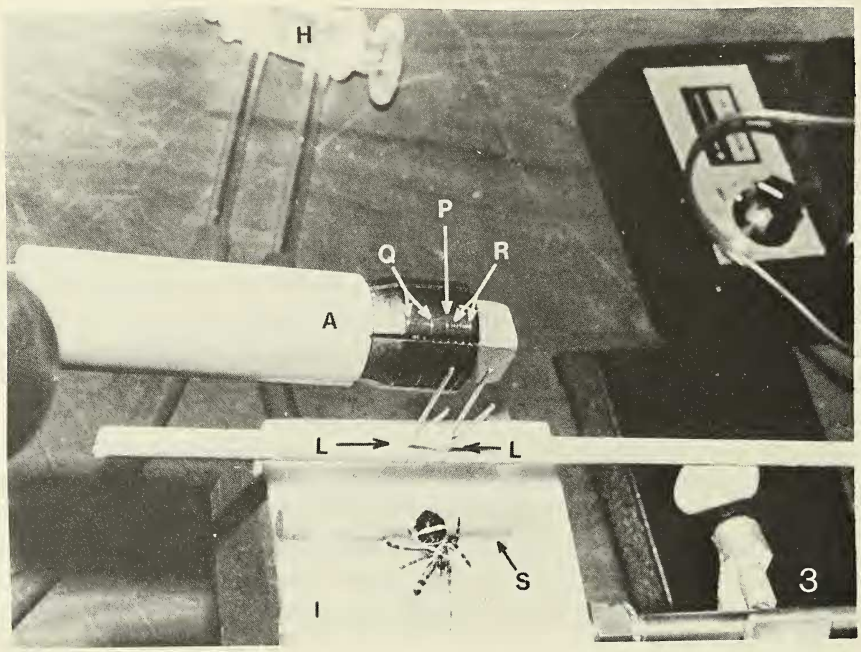


Fig. 3.–Spider, *A. argentata* pinioned ventral side up, with major and minor ampullate pairs of silk fibers being wound on the expandable mandrel.

have taken place, it is advisable to move a continuing sample to an unused section of the mandrel. Since an observer must remain watching the operation, with one hand on the control switch of the motor driven mandrel for as long as twenty or more minutes, it is highly desirable that here, as in the pinioning step, a second person should be available to alternate between note taking and observing.

A bundle of fibers wound onto an untapered mandrel cannot be removed, except by cutting it free. This may be a satisfactory solution in some cases. But a toroidal bundle of a continuous fiber or pair of fibers is essential for certain operations, and in any case, may be manipulated with ease, as compared with the same bundle that has been cut at one point. An expandable mandrel makes the former possible. In operation the slotted section of the mandrel is expanded before the bundle sample is to be wound on it. After it has been accumulated and its lead-in backwound, the expanding plug is removed (a wrench on it and a second on a flattened section of the mandrel will be required). To facilitate removal of the sample and aid in its subsequent manipulation, it has been found to be useful to provide it with "handles." These are conveniently made from continuous filament nylon sewing thread. Cotton or any other non-continuous filament thread should not be used, since these may provide fiber fragments as contaminants. Colored nylon thread provides contrast and, if desired, species can be identified by using a different color for each. To make the handle, the end of the nylon thread is passed into the aperture normally occupied by the expanding plug (now removed), up through the slot required to provide for expansion, over the bundle and knotted in a convenient loop. A double knot should be used since nylon knots are apt to slip. Two of these loops, are ample to allow for handling without the need for the investigator to touch the sample. One of the loops may be allowed to have a long end, to which an identifying label may be attached.

With the loops in place, the bundle is urged toward the outer end of the mandrel by means of a hooked micro dissecting needle, again making use of the slots as openings. It is necessary to do this by very small individual movements of the bundle, going around it from slot to slot. Any attempt to force it will run the hazard of fiber breakage and subsequent tangling and snarling. At the very end of the removal the nylon thread loops are grasped and separated, at 180° to each other, by one operator. The other operator completes the shift of the fiber bundle off the mandrel, while the one holding the loops keeps them far enough apart to prevent the bundle from snapping into a convoluted "muff" at the instant of relaxed strain. A common laboratory glass desiccator, without desiccant, provides a useful means of storing the samples, to prevent contamination by particulate matter in the air.

APPARATUS

An apparatus for forcible silking consists of three essential elements: 1) a variable speed drive, 2) a rotating take-up mandrel, and 3) means for traversing the filament as it is wound. Desirable features include compactness, easy accessibility and versatility.

As illustrated in Figure 4 the apparatus consists of a 10″ square base which supports front and side panels 10″ high. Although a totally enclosed cubical box could be employed, the open structure facilitates access to the drive mechanism as necessary. Mounted on the front panel is a Minarik model SL32 speed control designed specifically for operation with a Bodine-34 115 volt DC motor. The control provides two speed ranges which are infinitely variable by means of the centrally mounted control knob. Although normal

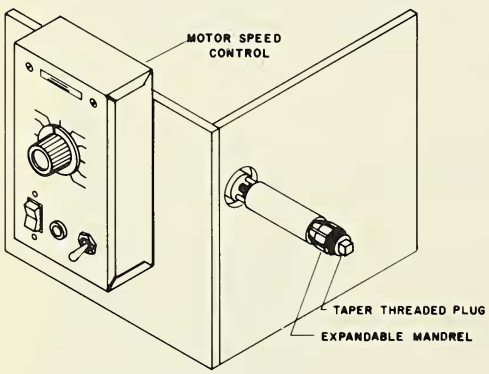
operation is forward, with the spindle moving clockwise, the control provides reverse rotation capability. A run-stop switch located at the lower right corner of the control allows the operator to start and stop the spindle by touch while closely observing the spider through the microscope.

Protruding through a 1½″ diameter opening in the side panel is the spindle-mandrel assembly onto which the silk filaments are wound. Two types of mandrels are used, one having an expandable portion at the end, and the other being a plain cylinder with a black anodized surface. Both mandrels are 1″ diameter and 4″ long.

As illustrated in Figure 5 the motor shaft is connected to a countershaft by means of a ¼″ pitch chain. Since fractional horsepower dc motors usually do not rotate smoothly at very low speeds a two-stage speed reduction of 4 to 1 from motor to spindle shaft is provided by suitable sprockets. A 20 tooth sprocket on the motor shaft drives a 40 tooth sprocket on the countershaft. The 10 tooth sprockets on the end of the countershaft drive two sprockets mounted on the spindle shaft. One of these is a 21 tooth sprocket which is free running on the spindle shaft. The other sprocket has 20 teeth and is locked on the spindle shaft by means of a set screw. A flat, milled into the spindle shaft, permits the set screw to be tightened without deforming the cylindrical surface of the shaft.

Inserted in the hub of the 21 tooth sprocket are two 1/8″ diameter steel rods which extend into coresponding holes in the mandrel. The end of the spindle shaft is threaded, (3/8-24-NF), and the mandrel screws onto the shaft as shown.

In operation, the spindle shaft is direct driven by the motor drive when the 20 tooth sprocket is locked to the spindle shaft. The free-running 21 tooth sprocket rotates at 95% of the speed of the spindle shaft. Since the free-running sprocket drives the mandrel 5% faster than the speed of the spindle shaft, the mandrel is thereby gradually advanced outward on the threaded portion of the spindle shaft. For each turn of the spindle shaft the mandrel advances 5% of the lead of the screw thread on the spindle shaft. As a result, the lead of the spider silk helix is 5% of the 3/8-24 NF thread, or 0.05 x 0.0417″, which is about 0.002″. This makes it possible to wind an evenly spaced helix approximately 40



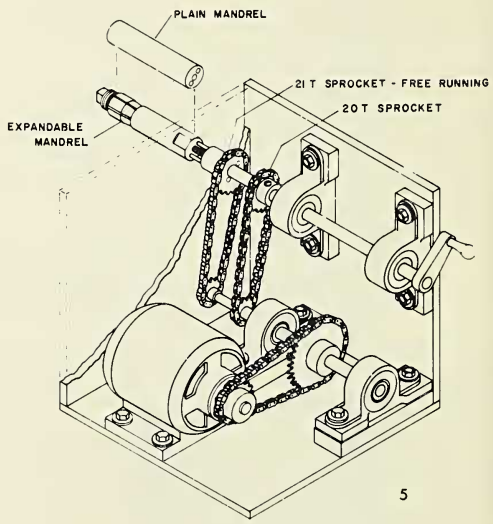


Fig. 4.—Apparatus for the forcible silking of spiders.

Fig. 5.—Drive mechanism of the apparatus.

m long onto each axial inch of mandrel, which at about 2 m/min, will require somewhat more than 13 minutes. If conditions are changed during silking, it is only necessary to move the silk guide needles (Fig. 2, L) to the left so as to provide an obvious gap to denote the new situation. To set the mandrel in starting position it is merely necessary to loosen the set screw in the hub of the 20 tooth sprocket so that the spindle shaft may be screwed back into the mandrel by means of the hand crank, while restraining the mandrel from rotation. The set screw should then be re-tightened.

In the event that it is desired to wind a silk bundle, rather than a helix, the set screw may be loosened and there will then be no mandrel advance. In this condition, drive to the mandrel will be through the 21 tooth sprocket and the 20 tooth sprocket will be free-running.

To provide the capability for diameter reduction of the mandrel, thus facilitating removal of tightly wound bundles, an expandable mandrel is desirable. This device is fitted with a ½″ NPT pipe plug threaded into the outer end of the mandrel, which is slotted to provide for the expansion caused by the tapered pipe plug. Prior to winding, the plug is screwed all the way into the mandrel. When winding is complete the mandrel can be prevented from rotating by being held in the jaws of a wrench, using the flats, while another wrench is employed to remove the pipe plug thus allowing the mandrel to contract.

DISCUSSION

Although the apparatus described in this paper was developed and has been used for the forcible silking of spiders, its versatility may well be adapted to the silking of other silk-producing animals. In this connection, an axiom from the field of macromolecular chemistry of linear, fiber-forming molecules must be kept in mind. It is known that strong fibers cannot be made by simple extrusion. Molecular segments must be oriented by stretching (technically called drawing) the macro structure during some phase of the production of the fibers. An example would be the drawing out of the progenitive polypeptide by the side to side wagging motion of the larva of *Bombyx mori*, as it forms its cocoon. It has been brought to the attention of one of the authors (RWW by Ms. Lottie Spainhour) that when *B. mori* is in the "cocoon-ready" stage, silk may be forcibly drawn from it. The same may be possible with other members of the same order. If such would be the case, using the described apparatus, it might be possible to secure samples of silk under controlled conditions in the laboratory, rather than from unravelled cocoons or in the field.

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